

Aspects of breeding morphology in *Mertensophryne micranotis* (Anura: Bufonidae): secondary sexual characters, eggs and tadpole

Alice G. C. Grandison

Department of Zoology, British Museum (Natural History), London SW7 5BD

Introduction

A few years ago while examining the holotype and paratype of the East African dwarf toad *Mertensophryne micranotis* (Loveridge), series of tiny, conical, unpigmented spines, some of which appeared to be inward curving, were noticed on the puckered rim and inner surface of the vent in the male paratype. Loveridge (1925) described the specimen, which measured 22 mm in snout to vent length, as having been taken *in coitu* with the slightly larger (23 mm) female holotype. However he made no mention of these curious vent structures. Nor did he describe the cluster of heavily cornified spines on the male's thumbs which are quite unlike the thumb pads of any other African bufonid, except the Mount Nimba viviparous toads, *Nectophrynoides liberiensis* Xavier and *N. occidentalis* Angel. The vent of other adult male *micranotis* available to the author at that time was not similarly modified.

Because the vent skin of the male paratype had been damaged by having had a pin driven through it and because there was a slight possibility of the spines being of no sexual significance a report of this author's observation was delayed pending the collection of additional sexually mature individuals.

Recently further material from forested coastal areas of Tanzania and Kenya have been made available for study. The Kenyan material comprises adults and larvae obtained from crevices in rotten trees 1.5 m from the forest floor and further adults from *Achatina* shells. The collector was attracted to the tree hole by faint high pitched squeaking (A. Duff-Mackay, pers. comm.). Although the material now available contains several males with fully developed thumb asperities only two of the recently acquired specimens also have spines on the vent. One of the two males was found with a gravid female in a tree cavity on 18 May 1971. It is conjectured that these vent structures probably develop only at the peak of spermatogenesis and regress shortly after mating and while the thumb asperities are still cornified. If this is so, then it is reasonable to assume that they function as a coupling device, perhaps enabling the male to maintain a tenacious grip on the female's vent during mating. Because a coupling device would be superfluous in species practising external fertilisation its occurrence in *micranotis* could indicate that the eggs in this species are internally fertilised.

By dissecting the cloacal tube and with the aid of interference contrast microscopy the spines were revealed as being restricted to the ridges of the puckers of the vent; none lies in the furrows. There appear to be approximately three tiers of spines, the outermost on the rim of the vent, the others internally at the entrance to the cloacal tube. All are orientated towards the lumen but directed somewhat posteriorly, Fig. 2.

It is suggested that in amplexus the male's spines slot into the furrows of the corrugations of the female's orifice. If they are supplied with a capillary network they may become erectile during amplexus, thus increasing the male's penetration of the female's vent, as well as his area of attachment to his mate. No pores, papillae or setae or other external structural modification suggesting a sensory function were detected by stereoscanning the spines. The presence, position and orientation of the spines are interpreted as a modification for ensuring a close fastening of the vents of the two sexes during insemination and preventing loss of sperm.



Fig. 1 *Mertensophryne micranotis*: A, dorsal view of subadult A/1270/1 $\times 3$; B, spines surrounding the vent of a sexually mature male A/1150/2 $\times 5$.

Eggs and internal fertilisation

In an attempt to test the hypothesis of internal fertilisation, eggs of two gravid females, one of which (the holotype) was taken in amplexus, were examined but no evidence of cell division was found. In the case of the holotype, such evidence would be unlikely for if sperm had entered the female the interval between the event and the female being killed and preserved could have been insufficient for the sperm to have reached the ova. The non-typical female may not have mated or, if inseminated, its eggs like those of the holotype may not have had time to undergo cell division. The eggs on one side of one female were removed, measured and counted. They are in three size classes. The largest class (av. diam. 1.8 mm) are lightly pigmented at the animal pole and number 16, the middle class (av. diam. 1.0 mm) are more heavily pigmented and number nine. The smallest ova are uniform white and only 0.4 mm in diameter. The total complement of eggs is estimated at 76.

The eggs and amplexus in other African bufonids

Only five other species of African bufonids have been found to contain clutches of less than 100 pigmented ova. Schmidt & Inger (1959) quote egg counts of 31 and 35 large ova in two females of their new species *Bufo melanopleura*, which measured 22.2 mm SVL and 22.7 mm SVL respectively. In addition each ovary had as many small immature ova as mature ones. Although Tandy & Keith (1972) report the clutch size in *Stephopaedes anotis* (Boulenger) as 22 and attribute this count to Poynton (1964), in fact Poynton cites 85.

Novak & Robinson (1975) provide data on the average clutch size and egg diameter for a number of species of *Bufo*, including a few African ones, but repeat the error made by Power

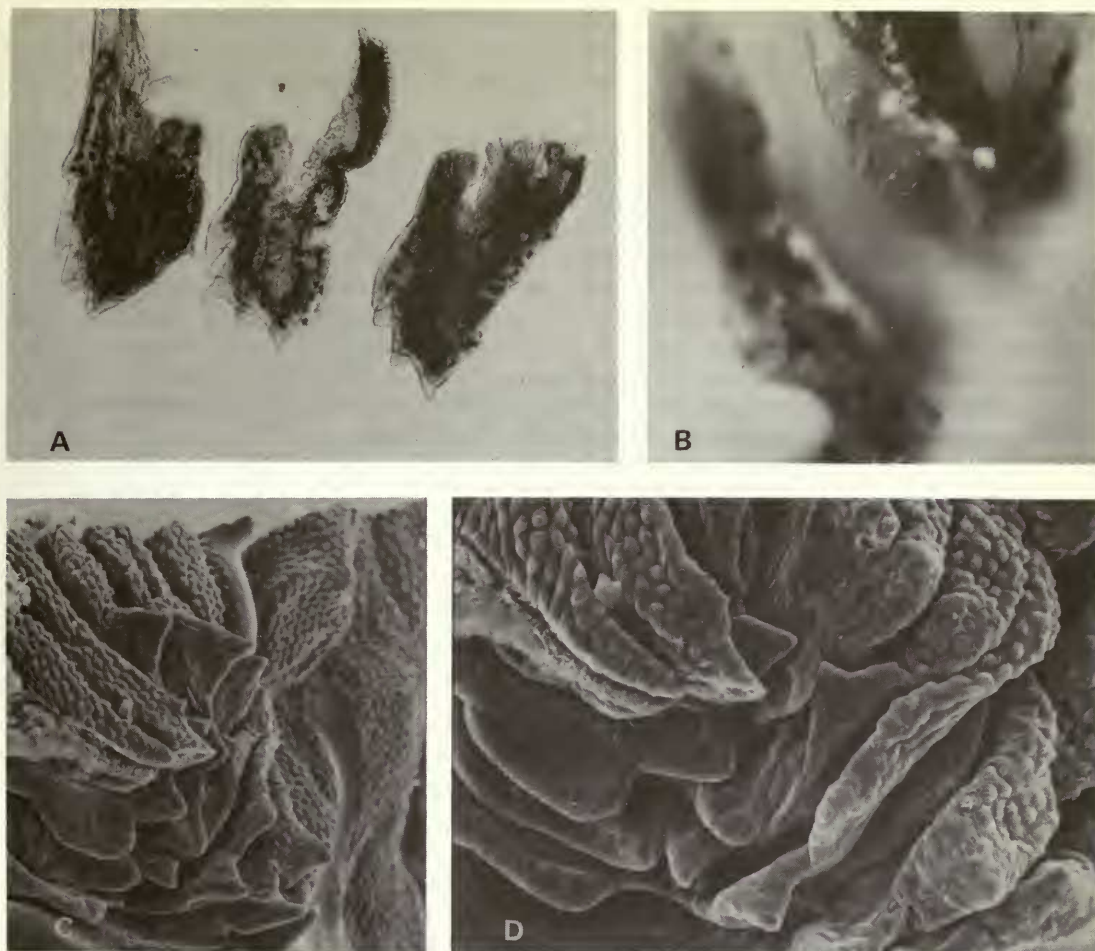


Fig. 2 *Mertensophryne micranotis* sexually mature male: A, three ridges of vent skin dissected and laid flat to illustrate projecting spines on medial surface, $\times 6$; B, sagittal section of the cloacal tube showing spines on lining of the tube and at its aperture, $\times 10$; C-D, portions of median surface of vent skin, $\times 151$ and 250 .

& Rose (1929) who attributed a clutch of 7000 eggs to a 37.0 mm SVL *B. rosei* Hewitt. They do however emphasise in the text of their paper the physical impossibility of such a small toad accommodating such a vast number of eggs. In the course of the present study the total number of enlarged heavily pigmented ova was counted in a 30.0 mm SVL *Bufo rosei*; there were 90 and the diameter of the eggs averaged 2.5 mm. The two ovaries contained about 50 very small unpigmented ova as well. A similar clutch size was found in *Bufo tradouwii* Hewitt. *B. rosei* and *B. tradouwii* have recently been referred to a new genus (Grandison, 1980). The internally fertilised *Nectophrynoides malcolmi* Grandison has the smallest clutch of pigmented eggs (11–31 : M18). Of the six species the amplexic position is unknown in all except *N. malcolmi* in which the male has been reported as assuming a belly to belly position with the female while in lumbar amplexus (Grandison, 1978) and *B. rosei* which Power & Rose (1929) described as being axillary.

If, as the Kenyan material of *M. micranotis* suggests, the species' breeding site is restricted to crevices in rotten trees obtaining proof of the mating position will be difficult. An amplexic pair will have to be carefully winkled out without dislodging the male from the female but if care is exercised it should be possible to extract them by flooding the hole. By

maintaining the pairs in captivity for a short time and observing them carefully the function of these cloacal structures may be determined and examination of the eggs and reproductive tract may yield information on whether internal fertilisation is accomplished in *M. micranotis*.

The breeding behaviour and mode of fertilising the eggs remain unknown in numerous species of frogs and toads, particularly the small secretive species. Among African bufonids internal fertilisation has been demonstrated solely in five species of *Nectophrynoides* that produce their young by various forms of direct development, ranging from a terrestrial non-feeding tadpole to true viviparity (Grandison, 1978; Wake, 1980). In one of these five species (*N. occidentalis*) transfer of the sperm to the female is achieved by close apposition of the cloacal openings while the mating pair are in lumbar amplexus (Angel & Lamotte, 1948; Lamotte & Xavier, 1972). But none of these five species of *Nectophrynoides* has any apparent modification of the skin surrounding the cloaca that would lock the vents together during sperm transfer. Investigation of their cloacal muscles may however reveal significant differences from the arrangement in externally fertilised species.

Description of tadpole

Length of stage 30 of Gosner (1960) 13.1 mm. The nostrils and the eyes lie in a saucer-like depression which slants steeply forwards and downwards and is partially surrounded by a raised ring of slightly pigmented tissue, the outer rim of which is incomplete posteriorly where it merges with the trunk (Fig. 3B). Eyes situated at the posterolateral corners of the depression, partly obscured in dorsal view by the overhang of the crown. Orbitonasal line clearly visible. Spiracle single, sinistral, situated 65% posteriorly along trunk, its opening vertically oval, visible laterally. Vent median. Fig. 3C.

Oral disk approximately 40% of head width at level of disk. Rostral papillae absent. No gap in mental papillae. Suprarostrodont and infrarostrodont serrated and keratinized. Keratodonts compound. Keratodont formula I/II (Fig. 3A). Tail with dorsal and ventral fin margins parallel except distally where maximum height increases slightly. Tail tip broadly rounded. Tail appreciably lower than trunk. Axis of tail straight, in line with body axis. Tail length: head and trunk length approximately 2 : 1. Trunk height: height of caudal muscle at tail base 2.5 : 1. Head within 'crown' pigmented, trunk more heavily pigmented, tail unpigmented (Fig. 3C).

Comparison with other African bufonid tadpoles

Stephopaedes anotis and *Mertensophryne micranotis* are the only species of Bufonidae known to have a 'crown' and a continuous fringe of mental papillae with no median gap. A mental gap is characteristic of *Bufo* tadpoles (Channing, 1978). The tadpole of *micranotis* differs from that of *S. anotis* in total length, in having an infrarostrodont, in fewer keratodonts with no interrupted supra-angular series, in tail shape and length and in the less pronounced and incomplete development of the 'crown'. The osteological and external morphological differences between adults of these species will be discussed in a forthcoming paper (Grandison in prep.).

Function of the larval 'crown'

The tadpoles of *Stephopaedes anotis* were described by Channing (1978) as having been found in stagnant pools in tree buttresses and were seen to be clinging to slimy bark in a tail-down position. He suggested that the function of the head 'crown' may be to exclude from the eyes and the nostrils the scum accumulating on the water surface. A similar function may be attributed to the convex ring of tissue on the head of the tadpole of *micranotis*. An alternative hypothesis is that the 'crown' may act as a sucker attaching the angled head to the

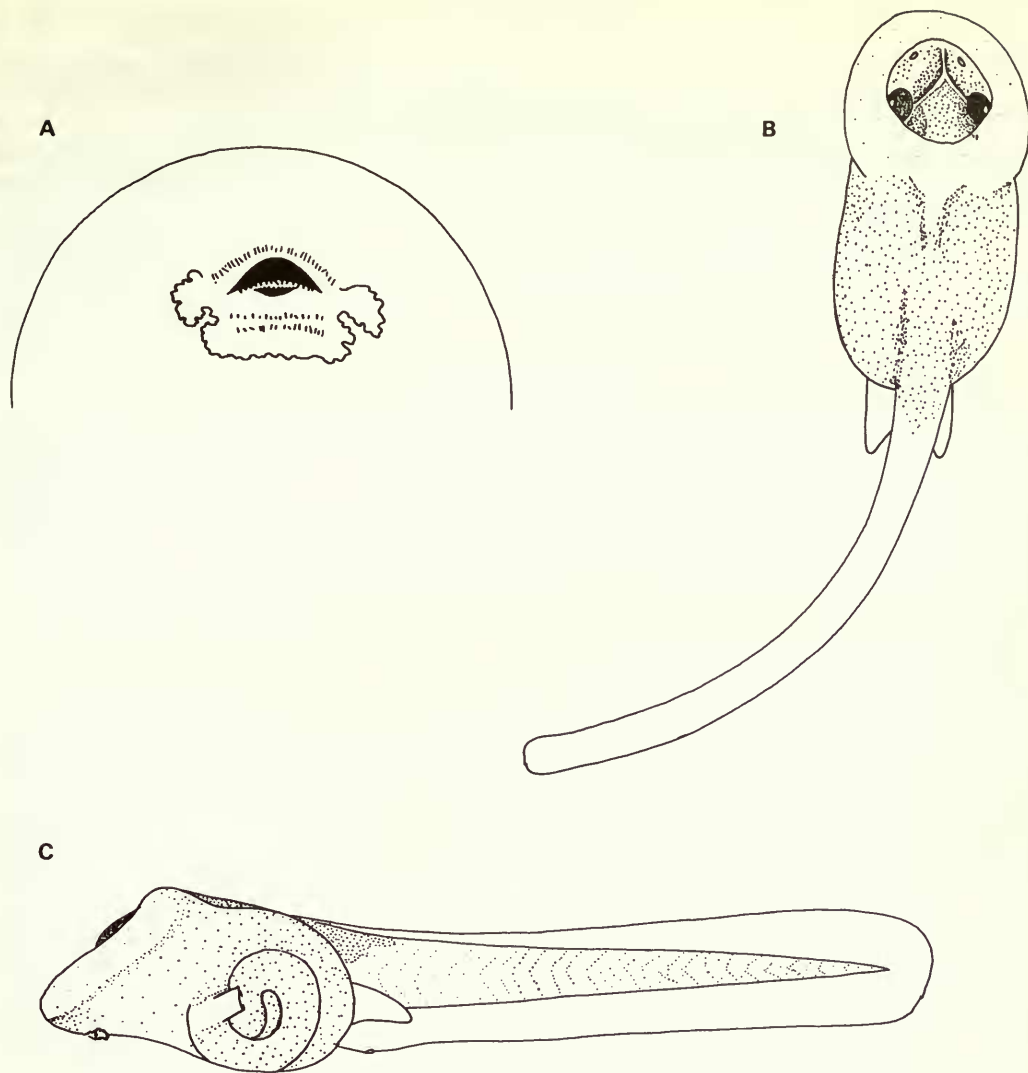


Fig. 3 *Mertensophryne micranotis* stage 30 tadpole: A, mouthparts; B, dorsal view showing the 'crown' surrounding the eyes and nostrils; C, lateral view showing the angular head, ventral mouth and long tail.

lining of the tree hole and allowing the ventrally placed mouth to browse on algae at the base of the hole but this would imply that the tadpole feeds head downwards. Until further field observations on the behaviour of the tadpole and the breeding site become available the function of the crown remains purely speculative.

Many questions relating to the life history and behaviour of this species remain unanswered. Some of them will be answered when amplexic pairs are obtained and fully ripe eggs available. Others, such as does the species select as breeding sites tree holes that are exposed to the elements and fill with water, what are its special requirements on tree hole size and aperture, height of hole from forest floor, type of tree and diameter of its trunk, may necessitate a longer period of field study. It is hoped this note will stimulate an interest in patterns of breeding behaviour in frogs and toads, especially in the small East African toads about which so little is known.

Acknowledgements

The assistance given by Mr R. C. Drewes (California Academy of Sciences) and the ready co-operation, generosity and field notes supplied by Mr A. Duff-MacKay (National Museums of Kenya) are gratefully acknowledged. Study material was made available by Mr Duff-MacKay, Dr E. E. Williams (Museum of Comparative Zoology), Dr A. E. Leviton (California Academy of Sciences), the late Dr C. F. Walker (Museum of Zoology, Michigan), Dr K. M. Howell (University of Dar es Salaam).

The author's sincere thanks are also extended to her colleagues Dr H. M. Platt and Mr D. Claugher for assistance with interference contrast microscopy and to Dr J. D. George for his advice.

Materials examined

28 adults and subadults: MCZ 10333 (holotype of *M. micranotis*), MCZ 10334 (paratype of *M. micranotis*), MCZ 25090 (holotype of *M. micranotis rondoensis*), MCZ 25091-94 and MCZ 25098-99 (paratypes of *M. m. rondoensis*), MCZ 32107, MCZ 12455, MCZ 26668, CAS 89737, UMMZ 61367, BM 1947.1.1.51-53, BM 1951.1.8.28, BM 1978.613, A/1150/1-4, A/1265, A/1266, A/1267, A/1268/2, A/1270/1.

28 tadpoles (stages 30-45): A/1150/5-9.

Abbreviations

A	National Museums of Kenya
BM	British Museum (Natural History)
CAS	California Academy of Sciences
MCZ	Museum of Comparative Zoology
UMMZ	Museum of Zoology, University of Michigan

References

- Angel, F. & Lamotte, M. 1948. Nouvelles observations sur *Nectophrynoides occidentalis* Angel. Remarques sur le genre *Nectophrynoides*. *Ann. Sci. nat. Paris* **10** : 115-147.
- Channing, A. 1978. A new bufonid genus (Amphibia: Anura) from Rhodesia. *Herpetologica* **34** : 394-397.
- Gosner, K. L. 1960. A simplified table for staging anuran embryos and larvae with notes on identification. *Herpetologica* **16** : 183-190.
- Grandison, A. G. C. 1978. The occurrence of *Nectophrynoides* (Anura Bufonidae) in Ethiopia. A new concept of the genus with a description of a new species. *Monit. zool. ital. N.S. Suppl.* **XI** : 119-172.
- Grandison, A. G. C. 1980. A new genus of toad (Anura : Bufonidae) from the Republic of South Africa with remarks on its relationships. *Bull. Br. Mus. nat. Hist. (Zool.)* **39** (5) : 293-298.
- Loveridge, A. 1925. 31. Notes on East African Batrachians, collected 1920-1923, with the description of four new species. *Proc. zool. Soc. Lond.* : 763-791.
- Lamotte, M. & Xavier, F. 1972. Les amphibiens anoures à développement direct d'Afrique. Observations sur la biologie de *Nectophrynoides tornieri* (Roux). *Bull. Soc. zool. Fr.* **97** : 413-428.
- Novak, R. K. & Robinson, D. C. 1975. Observations on the reproduction and ecology of the tropical montane toad *Bufo holdridgei* Taylor in Costa Rica. *Rev. Biol. Trop.* **23** : 213-237.
- Power, J. H. & Rose, W. 1929. Notes on the habits and life-histories of some Cape Peninsula Anura. *Trans. R. Soc. S. Afr.* **17** : 109-112.
- Poynton, J. C. 1964. The Amphibia of Southern Africa. *Ann. Natal Mus.* **17** : 1-334.
- Schmidt, K. P. & Inger, R. F. 1959. Amphibians exclusive of the genera *Afraxalus* and *Hyperolius*. *Explor. Parc natn. Upemba Miss. G.-F. de Witte.* **56** : 1-264.
- Tandy, M. & Keith, R. 1972. 9. *Bufo* of Africa. In: Blair, W. F. (ed.), *Evolution in the genus Bufo*. Univ. Texas Press, Austin and London. viii + 459 pp.
- Wake, M. 1980. The reproductive biology of *Nectophrynoides malcolmi* (Amphibia: Bufonidae), with comments on the evolution of reproductive modes in the genus *Nectophrynoides*. *Copeia* no. 2 : 193-200.